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Commentary

Genomic profile of sporadic colorectal cancer liver metastases versus primary tumors as defined by high-density microarray techniques

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Currently, it is estimated that 90% of all sporadic colorectal cancer (sCRC) related deaths are (directly or indirectly) related to metastatic dissemination of the tumor (1). In fact, resection of localized lesions from primary non-metastatic tumors is associated with a favorable outcome, while in cases in whom metastases have occurred, complete cure is unlikely (2).

It is now well-established that the development and progression of any neoplasia is associated with an accumulation of

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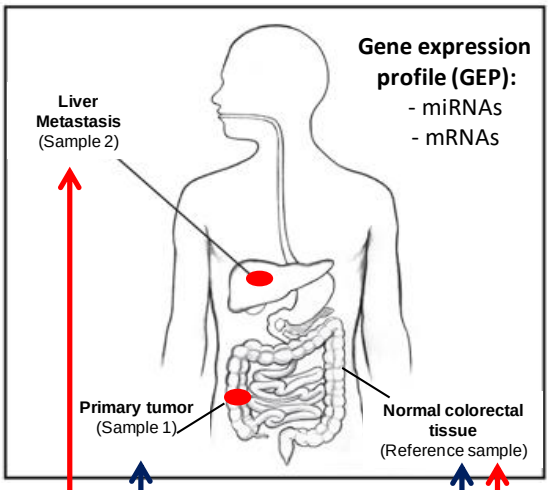
tissue-specific and tumor-associated genomic alterations (3). In recent years, important advances have been achieved in sCRC tumors as regards the identification of their specific chromosomal abnormalities, as well as the precise patterns of intratumoral clonal evolution pathways associated with the metastatic process (4,5). Interestingly, primary tumors and their paired metastases frequently show many deregulated genes in common (6-9), suggesting that (e.g. liver) metastatic lesions originate from a tumor cell clone which is already present in the primary sCRC tumor and very closely related to it at the genomic level. However, the precise molecular changes that are associated with the metastatic process still remain to be completely identified. Therefore, identification of those specific genetic/genomic alterations that could be detected already at diagnosis and identify patients who are at risk of harboring (or developing) metastases, might significantly contribute to the development of new and useful strategies for the diagnosis and management of sCRC patients.

In the past, we have described those chromosomal abnormalities that are present in primary tumors from patients with metastatic vs. nonmetastatic sCRC, (10,11) using fluorescence *in situ* hybridization (FISH) and single nucleotide polymorphism (SNP) arrays (10,11). Briefly, FISH revealed several numerical (gains or losses of a whole chromosome) and structural chromosomal abnormalities, including del(17p) and del(22q), to be highly prevalent among patients with primary sCRC who had synchronous liver metastasis (11). In addition, high-resolution SNP-arrays provided further detailed information on those genetic alterations that are most frequently associated with metastatic sCRC. Among other alterations, these included numerical gains of chromosomes 8q, 13q, and 20q and losses of the 1p, 8p, 17p, 18q, and 22q chromosomal regions (10). More recently, several gene expression profiles (GEP) have been identified as predictors for stage II CRC patient outcome, using the Oncotype DX® Colon Cancer test (Genomic Health, Inc., Redwood City, CA) (12) and Coloprint® (Agendia, Inc., Irvine, CA) gene chips, among other gene expression profiling (GEP) platforms (13). However, once again, the molecular mechanisms underlying the association observed between such genomic profiles and metastatic colorectal cancer, remain largely unknown.

Sayagués *et al* investigated the molecular heterogeneity of sCRC based on simultaneous assessment of the overall GEP of both coding mRNA and non-coding RNA genes -including miRNA, small nucleolar and large intergenic RNAs-; in a group of 23 primary sCRC tumor samples and their paired liver metastases (14). To the best of our knowledge, so far, few studies have systematically analysed resected human metastatic tissues *vs.* their corresponding primary tumors, plus the associated normal colorectal tissue from the same patient (Figure 1) (7). Overall, the metastatic tumor samples analysed showed a GEP that was highly similar to that of their paired primary tumors. In fact, liver

metastases systematically showed deregulated transcripts of those genes identified as being also altered in their paired primary colorectal carcinomas. Thus, both the primary tumors and their paired liver metastases showed overexpression of the FOXQ1, MMP7, CLDN1 and TACSTD2 mRNAS and the miR-4417, miR-503, miR-1290, miR-3687, miR-183, miR-224 and miR-1246 miRNAs, together with downregulated expression levels of the CLCA4, CA1, AQP8, ZG16, GUCA2B and SLC26A3 mRNAs and the amount of the miR-215, miR-133a, miR-375, miR-133b and miR-138 miRNAs. Despite this, several coding and a fewer non-coding RNA transcripts were found to be specifically deregulated in liver metastases while expressed at normal levels in their paired primary tumors, which might potentially reflect adaption of the tumor cell to the liver microenvironment. Newly deregulated metastatic transcripts included overexpression of the APOA1, HRG, UGT2B4, RBP4 and ADH4 mRNAs and the miR-3180-3p, miR-3197, miR-3178, miR-4793 and miR-4440 miRNAs, together with decreased expression of the IGKV1-39, IGKC, IGKV1-27, FABP4 and MYLK mRNAS and the miR-363, miR-1, miR-143, miR-27b and miR-28-5p miRNAs. In addition, specific comparison between the GEP of liver metastasis and their paired primary tumor samples (paired analysis) revealed 52 mRNAS (14 down-regulated and 38 up-regulated genes) and two (over-expressed) miRNAs (miR-122 and miR-4322) to be differentially expressed between the two tumor tissues. If such results are confirmed in an independent series of metastatic sCRC the identified molecular markers for metastatic tumor might potentially be used to develop a multi-marker prognostic test (15). Interestingly most of the proteins (i.e.:MMP7, TACSTD2, CTHRC1 and KRT23) coded by this set of 52 differently expressed mRNA genes, have been found to be secreted and thereby present, both in tumor tissues and the plasma of CRC patients (16). These later observations not only demonstrated secretion of these proteins outside the tumor cell, but they also point out the potential utility of these genes as serum biomarkers for early diagnosis and monitoring of CRC patients.



1ST GEP analysis

2nd GEP analysis

Final GEP analysis

Top deregulated transcripts exclusively in liver metastases

mRNA	miRNA
⇓ COL12A1	⇑ hsa-miR-572
⇑ DEFB1	
⇓ PTGER3	

Top deregulated transcripts in primary tumor

mRNA	miRNA
⇓ CLCA4	⇓ hsa-miR-215
⇓ CA1	⇑ hsa-miR-31
⇓ SLC4A4	⇑ hsa-miR-4417
⇓ AQP8	⇑ hsa-miR-503
⇓ ZG16	⇓ hsa-miR-139

Top deregulated transcripts in liver metastases

mRNA	miRNA
⇓ CLCA4	⇑ hsa-miR-122
⇑ FGA	⇓ hsa-miR-133a
⇓ CA1	⇑ hsa-miR-4417
⇑ ALB	⇑ hsa-miR-1290
⇑ CRP	⇓ hsa-miR-133b

Figure 1: Gene expression profiles (GEP) of primary vs. metastatic colorectal cancer. Scheme representing mRNA and miRNA differentially expressed between the different types of samples analyzed: primary sporadic colorectal tumors *vs.* normal colorectal tissue, colorectal liver metastases *vs.* normal colorectal tissue and primary sporadic colorectal tumors *vs.* their paired liver metastases (*q*-values < 0.01).

In addition, Sayagués *et al* (9) also highlighted the activation of genes associated with the TGF-β signaling pathway in the metastatic tumors, -e.g. RHOA, SMAD2, SMAD4, SMAD5, SMAD6, BMPR1A, SMAD7 and MYC-; thereby, these genes also emerge as candidate genes to play an important role in CRC tumor metastasis. In fact, such findings have led to the speculation that TGFβ signaling might be responsible, at least in part, for the aggressiveness of metastatic sCRC tumors, by conferring a higher invasive phenotype, which is a prerequisite for the settlement and growth of distant metastasis (17).

In summary, Sayagués *et al* provide new insights about particular gene signatures potentially involved in sCRC metastasis also laying the path for the identification of novel biomarker candidates for both early diagnosis of sCRC tumors, and more efficient treatment and/or monitoring of CRC tumor metastasis.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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